6 SPECTROPHOTOMETRY

6.1 Objectives

6.1.1 Understand and explain the principles of ultraviolet (UV), visible (VIS), atomic absorption (AA) and fluorescence spectrophotometric measurements.

6.1.2 Understand the practice of UV/VIS spectrophotometry and the specifics of operation of the spectrophotometers at DFS.

6.1.3 Perform instrumental analysis of carboxyhemoglobin using a UV/VIS spectrophotometer.

6.1.4 Interpret results by thoroughly examining and explaining the instrument printout.

6.1.5 Understand the quality control aspects of spectrophotometric testing.

6.2 Estimated Time: Two weeks

6.3 Methods of Instruction

6.3.1 Lectures

6.3.1.1 Principles of spectrophotometry and spectrofluorometry

6.3.1.2 Components and operation of the UV/VIS spectrophotometer

6.3.1.3 Specimen preparation

6.3.1.4 Specimen analysis

6.3.1.5 Result interpretation

6.3.1.6 Palladium chloride diffusion confirmation test

6.3.1.7 Salicylate confirmation by VIS spectrometry

6.3.2 Literature Review

6.3.2.1 DU Series 600 and 7000 Spectrophotometer: *Basic Instrument Training Manual and Advanced Application Training Manual,* Beckman

6.3.2.2 Toxicology Technical Procedures Manual

6.3.2.3 Moffat, A.C., editor. *Clarke's Analysis of Drugs and Poisons*, 3rd edition. London: The Pharmaceutical Press, 2004 pp 313-327.

6.3.2.4 Willard, H. H., Merritt, L.L. Jr., Dean , J., Settle, FA., *Instrumental Methods of Analysis*, 7th Ed. 1988, Wadsworth Pub. Co., pp. 159-223.

6.3.3 Demonstration

6.3.3.1 The use of UV/VIS spectrophotometry for the quantitative analyses of carbon monoxide and salicylates will be observed from beginning to end and notes will be taken by the Trainee.

6.3.4 Laboratory Exercises

6.3.4.1 Analyze low, medium and high controls for the presence of carbon monoxide (CO).

6.3.4.2 Screen one batch of 5 blood specimens for the presence of CO. At least 2 of the specimens will be positive and at least one specimen will be negative.

6.3.4.2.1 Calculate the % saturation of each specimen.

6.3.4.3 Confirm the presence of CO using the palladium chloride diffusion test.

6.4 Evaluation

6.4.1 Written Examination

6.4.1.1 This will be administered as a “take home” exam.

6.4.2 Laboratory Competency Testing

6.4.2.1 A series of at least 5 previously analyzed blood specimens will be presented to the Trainee for CO analysis. The results obtained by the Trainee must agree within 20% of the previous results.

6.4.3 Courtroom Exercise

6.4.3.1 The Trainee must be capable of answering questions on this Module such as would be expected in a courtroom scenario.

6.5 Examination Questions

6.5.1 What are the wavelength ranges for visible and ultraviolet electromagnetic radiation?

6.5.2 Explain what effects a change in solvent might have on the spectrum of a solute.

6.5.3 Discuss why a change in the pH of a solution can be important when using UV for analysis.

6.5.4 List and discuss some common sources of error in spectrophotometric measurements.

6.5.5 Define the following terms: wavelength, absorbance, transmittance, excitation, emission, bandwidth and Beer's law.

6.5.6 In the quantitative carboxyhemoglobin analysis, explain deoxyhemoglobin, oxyhemoglobin, methemoglobin and carboxyhemoglobin.

6.5.7 How are the results reported on the certificate of analysis for CO?

6.5.8 Explain the principle of the palladium chloride confirmation.

♦ End